9-1-1991

Cytoplasmic Action In Development

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COMMENTARY

CYTOPLASMIC ACTION IN DEVELOPMENT

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A commentary on
CYTOPLASMIC ORGANIZATION SYSTEMS.
Edited by George M. Malacinski. McGraw-Hill Publishing Company, New York. $59.95. xxiv + 482 p.; ill.; index. ISBN: 0-07-039749-X. 1990.

THE ONLY way to get from genotype to phenotype is via development. This is an enormous span, covering such diverse events as the activation of gene transcription in the early embryo, the formation of tooth enamel, and the maturation of the B lymphocytes. Not surprisingly, then, there are some areas of developmental biology whose problems are predominantly genotypic (e.g., "How might transcription factors interact with chromatin to activate specific genes in certain cells?"), and other areas whose problems are predominantly phenotypic (e.g., "How might cell surfaces interact to form tissues and organs?"). Genotypic questions have provided most of the subject matter for developmental genetics, while phenotypic questions have provided the subjects for embryology, both descriptive and experimental. In recent years, the advances made by the genotypic side of developmental biology have been so spectacular that it sometimes is feared that the phenotypic perspectives might become lost.

Although the distinction between phenotypic and genotypic developmental biology is relatively new, the fear that developmental biology might be "taken over" by genetics is nearly as old as the separation of genetics from embryology in the 1920s. When experimental embryology separated itself from evolutionary problems at the turn of the century, its domain included the studies of inheritance, development, regeneration, and senescence. One of the largest questions for this newly organized field was: Which compartment of the zygote—the nucleus or the cytoplasm—directed heredity and development? In a series of interactions that lasted over two decades, E. B. Wilson and T. H. Morgan chased the evidence into the nucleus, although Morgan had thought at first that cytoplasmic factors determined all phenotypes. The path that led from experimental embryology into genetics was the X chromosome. Morgan and Wilson disagreed as to whether this nuclear entity actually controlled sex determination or whether it was a consequence of earlier, cytoplasmic, sex determining mechanisms. Eventually, Morgan and his coworkers correlated several inherited factors, as well as sex determination, to the X chromosome. In this way, the embryologist Morgan inadvertently created a new genetic science (Gilbert, 1978, 1987). Soon after 1911, genetics arose as a separate discipline, complete with its own techniques, favored organisms, rules of evidence, journals, and vocabulary. The remarkable success of genetics in the 1920s and 1930s caused it to become the preeminent way to study inheritance, and it redefined the other disciplines in genetic terms. The study of inheritance became genetics, which Morgan defined as the discipline concerned with the transmission of nuclear genes (Morgan, 1926). Morgan's exclusion of cytoplasm and development from the realm of inheritance was soon viewed as dogma (Sapp, 1987). Embryology was redefined as the study of changes in gene expression over time (Morgan, 1934),

The Quarterly Review of Biology, September 1991, Vol. 66, No. 3

0033-5770/91/6603-0004$01.00

and evolution was redefined as changes in gene frequency over time (Dobzhansky, 1937). Thus, evolution and embryology, which had traditionally been sciences of the phenotype, were given new, genotypic, definitions.

These new definitions originally went counter to the prevailing paradigms of these fields. Evolution had been the province of paleontologists who reconstructed ancient skeletons and phylogenies. Similarly, embryologists had not concerned themselves with questions of gene expression. The predominant problem of embryology from the 1700s through the 1950s was the creation of ordered form, morphogenesis, not differentiation (Haraway, 1976; Lenoir, 1982). The genetic redefinition of embryology collapsed the morphogenesis question into a subset of the differentiation question. To geneticist Richard Goldschmidt (1939, p. 1), this was axiomatic: "Development is, of course, the orderly production of pattern, and therefore after all, genes control pattern." Embryological morphogenesis, too, came to be seen as an epiphenomenon of differential gene expression.

While the geneticists were making their great discoveries into the mechanism of hereditary transmission, the embryologists were also having their own golden era. Ignoring genetics altogether, embryologists embarked on the program which Joseph Needham (1936) christened "*Gestaltungsgesetze*, the rules of morphological order." Here, the transplantation experiments of Spemann, the Mangolds, Holtfreter, Hamburger, Hörstadius, Harrison, Willier, and Rawles set new experimental standards for embryologists and provided astounding new insights into how organs were constructed. The concept of gene expression is absent in the major embryology books of the 1920s through the 1940s. Although experimental embryology had successfully separated itself from the earlier traditions of developmental anatomy, it remained a phenotypic science, and it identified itself as a science concerned with cytoplasmic changes. As Frank R. Lillie wrote in his critical review of 1927, "The germ exhibits the duality of nucleus and cytoplasm; the geneticist has taken the former for his field, the embryologist the latter."

The nuclear envelope, however, proved to be a permeable barrier. More and more, geneticists began to see differential gene expression as the cause for embryogenesis. Jumping over the nuclear boundary, they claimed embryology as part of their domain as well. Waddington, Schultz, Goldschmidt, and others began studying the mutations that altered the basic patterns of insect development, and Goldschmidt (1938) saw development as being identical with "physiological genetics." He claimed that *geneticists* must explain embryology because the embryologists were not capable of doing so. In a later statement that reflects this boundary dispute, Goldschmidt wrote (1955, p. 247) that "geneticists will continue to worry about the problem of genetic action and take the risk of climbing over the fence erected by some jealous embryologists, who, while claiming the kingdom for themselves, do not set out to till its soil." C. H. Waddington began reintroducing embryology into English-language genetics textbooks (1939) by stating, "Now that the mechanism of inheritance is known, in its main outlines at least, it is possible to tackle the next question, of how the genes affect the developmental processes which convert the fertilized egg into the adult organism." If the embryologists were not going to discuss embryogenesis in terms of gene activity, the geneticists would.

But embryologists had a strong research program of their own, and they did not like being told how to do their science. Ross Harrison, as chairman of the section of zoological sciences of the American Association for the Advancement of Science, addressed his colleagues (1937, pp. 8–9):

> Now that the necessity of relating the data of genetics to embryology is generally recognized and the "Wanderlust" of geneticists is beginning to urge them in our direction, it may not be inappropriate to point out a danger of this threatened invasion.
>
> The prestige of success enjoyed by the gene theory might easily become a hindrance to the understanding of development by directing our attention solely to the genom, whereas cell movements, differentiation and in fact all of developmental processes are actually effected by the cytoplasm. Already we have theories that refer the processes of development to genic action and regard the whole performance as no more than the realization of the potencies of the genes. Such theories are altogether too one-sided.

If these comments sound remarkably current, it is because this concern is still felt strongly among many contemporary embryologists, and it forms the basis for the volume *Cytoplasmic Organization Systems*, edited by George M. Malacinski.

But to return first to the history underlying these concerns, there were many arguments that embryologists brought against the genotypic approach to embryology. Some were eventually dismissed. For example, in the 1930s, most geneticists claimed that each gene was active in every cell. How, then, the embryologists would say, can you get differentiation to occur? Similarly, since most of the mutations that were analysed prior to the 1950s concerned modifications of adult structures, many embryologists claimed that the cytoplasmic organization systems (whatever they might be) formed the basic body plan of the organism, while the genes just put on the final secondary touches such as length of wing or bristle pattern. As embryologist E.E. Just (one of the most vociferous critics of genotypic embryology) decried, he was more interested in how the fly formed its back, not how it formed one of its dorsal bristles.

Other embryologists saw genotypic embryology as a new preformationism. N.J. Berrill (1941), who presided over the first Growth Society meeting (which was to become the Society for Developmental Biology), defined genes as "statistically significant little devils collectively equivalent to one entelechy." Similarly, contemporary developmental biologist Lauri Saxén (1973) has claimed that "Our present idea of progressive differentiation actually is not far removed from this classical homunculus concept. Thus, all the information required to build a complete organism is already present within the zygote and development is seen as a progressive expression of this genomic information" (p. 31). He satirized this view (Fig. 1) by comparing a "homunculus in the sperm as illustrated by the 16th century animalculists" with "the present view of the 'homunculoid' information in a germ cell." Oyama (1985) has also commented extensively on the similarity in the modern use of "genetic information" and "genetic program" with older concepts of entelechy and preformation. (Indeed, one cannot clone or make an antibody to the genetic program. No such thing exists. The genome is less like a programmed score than it is like an orchestra, wherein each member plays a single note and has perfect hearing. Upon hearing a certain phrase, a performer plays its note, which becomes part of a new phrase, et cetera.)

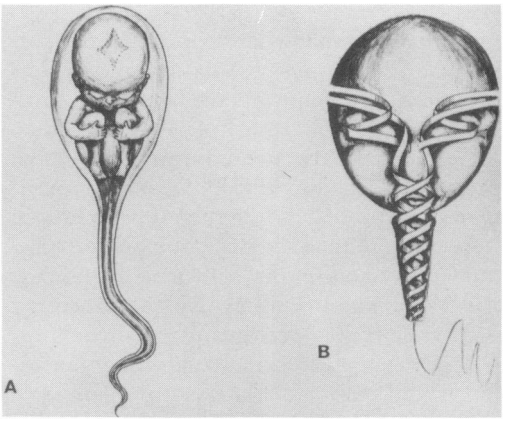

FIG. 1. HOMUNCULI FROM THE 16TH AND 20TH CENTURIES, DEPICTED IN SAXÉN, 1973.

Another of the criticisms of phenotypic embryologists against the genotypic redefinition of embryology has been that the one-dimensional chain of nucleotides that constitutes the inherited genome cannot construct a three-dimensional organism. After all, the genes can only specify proteins and RNAs. They can't of themselves make livers, teeth, and brains. Something else (i.e., a cytoplasmic organization system) must be present to organize the gene-encoded proteins. Foremost among these critics of genotypic embryology was Paul Weiss (1962). While acknowledging that cell differentiation was the product of differential gene expression, he saw the genes as reactive, rather than creative, molecules. They responded to a complex ecosystem of molecules that told them which genes to express at which times. Rather than being the control center or executive suite of the cell (to use two metaphors found in present-day textbooks), Weiss saw the genome more as a library or tool box.

To Weiss, it seemed easy to get from organism to molecules. Any good biochemist would be able to do this bit of analysis. What was difficult was the reverse trek, from molecules to organism. This synthetic task was

what the embryologist had to deal with. Knowing the genome alone would not be sufficient to determine how tissues and organs form, nor would knowing the component proteins and organelles of the cell. One had to know how these components interacted in the "molecular ecology" of cellular and supracellular structures. To dramatize this point, he presented a slide of a 16-day chick embryo under three conditions: intact, homogenized and fractionated (Fig. 2). No substance, he said, was lost or added during this procedure. All that disappears is the structural organization of the embryo. The easy problem is to catalog the molecules. The embryologist, however, must confront the problem of putting Humpty-Dumpty back together again.

Weiss's view that the genome was reactive rather than creative was shared by several holistically oriented embryologists, including Waddington. Waddington (1940) pointed out numerous embryological examples to show that the cytoplasm determined the nature of the cells, not the nucleus. First, in embryos such as tunicates and snails, the determination of cell fate is accomplished by the cytoplasmic region of the fertilized egg that the nucleus inherits during cell division. Second, in more regulative embryos, changing the region of cytoplasm in which a nucleus resides (as Driesch did in his pressure-plate experiments or Spemann did with hairloops) also changes its fate. Third, during induction, a substance made by one cell is able to alter the fate of a second cell by causing it to synthesize new products. Later, he would also cite Sonneborn's studies on *Paramecium* as demonstrating a "condition where it is the condition of the cytoplasm which determines which of the loci shall be in operation." Thus, in his 1956 book, *Principles of Embryology*, Waddington entitles his chapter on developmental genetics: "The Activation of Genes by the Cytoplasm." It is the cytoplasm that is active. The genes respond to it.

A fourth critique of the genomic approach to embryology has been that the genome cannot account for *all* differentiation processes, even some major ones. Waddington (1940), for example, saw that the environment determined whether any given *Bonnelia* larva was to become a large female echiuroid worm or its small symbiotic male. More

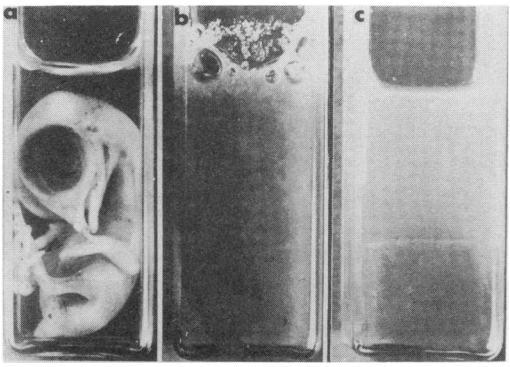

FIG. 2. WEISS'S CHALLENGE. A 16-DAY CHICK EMBRYO (A) INTACT, (B) HOMOGENIZED, AND (C) FRACTIONATED. (FROM WEISS, 1962).

recent evidence for the environmental causation of development includes antigen-dependent differentiation of B cells, experience-driven morphogenesis of neuronal connections, and temperature-dependent sex determination in fish and reptiles.

Until the advent of recombinant DNA techniques, there was an uneasy truce between the genotypic and phenotypic embryologists. The work of the phenotypic embryologists (written off by many of the genotypic school as being "classical"—i.e., outmoded—embryology) continued to analyse embryonic induction and morphogenesis; while the genotypic school (characterized as "irrelevant" by some of the phenotypic persuasion) sought answers to the questions of differentiation through the paradigm of differential gene expression. Neither had the physical or conceptual tools to explain all of development. Parratt (1988, p. 6) writes of Donald Brown in the early 1960s:

> Though no one at Carnegie was investigating biochemical aspects of embryology (the staff members were all classical or experimental embryologists), Brown had immediately warmed to the Department. He felt it was a place where he could do the sorts of experiments he wanted to do, "without anyone looking over my shoulder." He joined the Department as a research associate—the first of a new flock of embryologists that would slowly displace the old order.

Brown, who would later be the first to isolate

a vertebrate gene and characterize its differential transcription, is reported as saying that, at that time, the only thing he knew about embryology was that "it was a field so primitive that no modern research was being done in it."

As Boveri predicted in 1904, however, if there were only a way to isolate and amplify those genes responsible for development, then biochemists might be able to explain embryogenesis. The history of the molecularization of modern developmental biology has yet to be written, and there are several tributaries that converged to create the mighty stream. The techniques of nucleic acid hybridization and its offspring, recombinant DNA, obviously play a major role; so does the ability to use *Drosophila* as a developmental organism. Medical science also plays a large part in this story, since much of the funding for the molecularization of developmental biology came from its being thought medically relevant. The study of the human β-globin gene, critical for the treatment of clinical diseases such as sickle-cell anemia and β-thalassemia, gave us much of our knowledge concerning differential gene expression and promoter elements. The study of immunoglobulin gene regulation provided a wealth of information concerning enhancers; and cancer research provided a context to search for regulatory elements in any organism, even bacterial viruses.

One could not ask for more powerful examples of the genomic approach to developmental biology than the publications of 1990. In this one year, activin was seen as being the inducer of amphibian mesoderm (thus culminating a search begun with the transplantation experiments of Nieuwkoop in 1969), the murine *Steel* locus product, essential for the maturation of germ cells, hematopoietic stem cells, and melanoblasts, was identified (thereby culminating a series of investigations beginning with Russell in 1956), the mRNA for the bicoid protein was found to be sufficient to activate head formation in *Drosophila* (culminating the transplantation experiments that Sander began in the 1950s), and the SRY gene of the human Y chromosome was seen as carrying the major testis-determining determinant (possibly culminating a search that begins in human prehistory). In each ca the techniques of molecular biology had been used to answer a question posed — and left unsolved — by classical embryology.

In addition, the past four years has seen the transformation of at least two phenotypic-level embryology journals into genotypic-level embryology journals. The *Journal of Embryology and Experimental Morphology* changed its name to *Development* in 1987. Its editorial policy, while not excluding phenotypic development, is seen to favor more of the genotypic studies. More dramatically, the journal *Cell Differentiation and Development*, the official journal of the International Society of Developmental Biologists, underwent a striking metamorphosis to emerge as *Mechanisms of Development*. Although both old and new journals sought to understand the mechanisms by which development takes place, *CDD* looked at the level of cell and tissue interactions, while (the aptly abbreviated) *MOD* seeks papers that focus on the genomic control of differentiation. The change was announced in the final volume of the *CDD* (Publisher's note, 1990):

> The title of **Cell Differentiation and Development** will change as of Volume 33, No. 1 (December 1990) to **Mechanisms of Development (MOD)** to indicate the shift of its focus to follow the most exciting current research trends. The new Editors feel there is a demand for a journal that is dedicated solely to communicating molecular and genetic approaches to problems of developmental biology.

In addition, new journals such as *Genes and Development* also provide outlets for publications in genotypic developmental biology, while papers on the phenotypic side of development are often placed in zoology journals.

With all this success, then, we must ask: has the genotypic approach to development been successful in (a) totally submerging the phenotypic school, and (b) providing an adequate explanation for developmental phenomena. The essays provided in *Cytoplasmic Organization Systems* answer no to both these questions. Therein lies the importance of this volume of the Primers in Developmental Biology series, for it describes an embryology where the genes are secondary and reactive. Whereas two earlier volumes of this series, *Molecular Genetics of Mammalian Cells* and *Developmental Genetics of Higher Organisms*, stressed the genome as the active component, here the cytoplasm is given its due respect.

Brian Goodwin leads off the book with a thought-provoking essay on the relationship between gene action and morphogenesis. He finds that our paradigms of morphogenesis are based too greatly on the assumption that all morphogenesis can be explained by genes and their products. He finds that this assumption, though pervading developmental biology, is unproved and not inclusive. First, he notes the paradox that gene differences have not correlated well with morphological differences: some species that have greatly diverged morphologically have nearly identical sets of genes, whereas some species with very similar genomes have significantly different body types. But if gene products do not control development, what does? Goodwin proposes that the thermodynamics of reaction-diffusion mechanisms enable morphogenesis to occur. The substrates and products of such reactions are probably small molecules and ions (such as retinoids and calcium). The discontinuities in these products by the reaction-diffusion mechanisms forms the prepattern of the organism. Goodwin uses the analogy of gene products and metabolism to gene products and development. Just as the laws of thermodynamics determine which reactions are possible in a cell, the gene products are merely proteins that alter the rate of these reactions; so the gene products in development are merely the stabilizers of the already existing pattern produced by the reaction-diffusion mechanisms. "Just as gene products do not make metabolism possible, that being a result of the physical and chemical laws, gene products do not make morphogenesis possible, this also being a result of the laws of physics and chemistry" (p. 9). Goodwin quotes studies demonstrating that the reaction-diffusion mechanisms can predict the cleavage planes of the dividing egg, the transcription stripes of *Drosophila* segmentation genes, and numerous other patterns. The gene products that interact to produce these patterns are secondary stabilizers of the preexisting pattern formed by smaller molecules. Thus, in Goodwin's essay, we see morphogenesis without the nucleus. The genes exist merely to stabilize the cytoplasmic reactions that are a product of diffusion and the shape and size of the embryo.

In Gary Grimes's essay on the inheritance of cortical patterns in ciliates, we are confronted with a more concrete example of inheritance without genes. Back at the turn of the century, the field of heredity was larger than that of genetics. It included development and regeneration as well, and its paradigmatic organism was the flatworm, not the fruit fly (Fausto-Sterling and Mittman, in press). Jan Sapp (1987) has documented the hegemony of genetics over cytoplasmic theories of inheritance, and the only major surviving examples of cytoplasmic nongenic inheritance are found in the ciliates. Grimes provides an excellent review of the experimental evidence for cortical inheritance in ciliates such as *Paramecium* and *Tetrahymena*. These data are not necessarily new, nor are the interpretations. In fact, geneticists have long known about cortical inheritance but have marginalized it as an exception to the general rule of gene inheritance. Grimes claims that both DNA and cortical inheritance are subsets of "directed assembly" wherein the timing and placement of new structures are organized according to a template of preexisting structures. In the context of the cortical "mutants," where reversed cortical structures are stably inherited for hundreds of generations, the same gene products are assembled but differ in their organization. The real question is not the validity of cortical inheritance. Sonneborn, Nanney, and their colleagues have labored long and successfully to convince other scientists of its actuality. The question is whether protists are exceptions to the general rule or are part of the general rule. Are they "normal" or are they "exceptional"? Here Grimes does not meet the challenge, and merely states that since all membranes are mosaic structures and since new material is inserted into preexisting membranes, the facts of ciliates might be extrapolated to cells in general. But the fluid mosaic membrane of the metazoan is not the same as the highly structured cortical cytoplasm of the ciliate, and until stably inherited membrane structures are discovered in such metazoan cells (and not merely transient organizations such as those in compacting blastomeres), cortical inheritance is likely to remain at the periphery of discussions on inheritance and development.

Several of the chapters in this book concern

morphogenic determinants in oocyte cytoplasm. These chapters include research on carp, *Caenorhabditis*, insects, leeches, and mice. Here, the cytoplasm of a particular cell activates specific genes that give that cell its fate. While embryologists usually assume that most of vertebrate development depends on intercellular induction between adjacent tissues, Claudio Stern argues that there may be more cell lineage-type (mosaic) determination in vertebrates than had been expected. Indeed, Stern's chapter documents that the avian mesoderm cells are probably determined before passing through the primitive streak, and that the distinction between somite-forming mesoderm and nonsomitic mesoderm is likewise established by lineage and not by cell interactions.

Another group of chapters emphasizes the informational roles of cell surfaces and extracellular matrices in development. Paul Weiss had predicted that the "dynamic organization" of the embryo would become the central problem of development, and these chapters focus on the mechanisms by which the cell adhesion molecules, gap junctions, and extracellular matrices guide morphogenesis.

George Malacinski's preface and his essay coauthored with Anton Neff bring together several of these strands into a political statement concerning the importance of cytoplasmic systems. Knowing the entire nucleotide sequence of an organism, they write, still won't tell you how it develops, and evolutionary history may provide an organism with several ways of accomplishing the same events. Malacinski writes (p. xxii) that "development is too complex a process to be successfully comprehended by people who label themselves as being of this or that persuasion." In this, he is reiterating Berrill's charge to the first Growth Symposium meeting that development must be studied by combining the insights of numerous disciplines, including genetics, endocrinology, biochemistry, physiology, embryology, cytology, biophysics, mathematics, and even philosophy. Development is seen as a collection of disciplines, and would be cheapened by the hegemony of one method over all others.

The strength of this book is not in the individual essays. Much of this material will be familiar to developmental biologists, and some of it is out of date because of the long publication lag. But like Weiss's view of the organism, the volume is more than the sum of its component parts. Its strength lies in bringing together material from various developmental perspectives under a common banner. This is not a rear-guard reactionary book, but a volume that looks forward to the solving of developmental problems through a diversity of methods and viewpoints. It comes at an important time in the history of developmental biology. At the moment, the existing technologies and the Human Genome Organization zeitgeist of "your identity is your genes" are driving the equilibrium of developmental biology toward the genomic side. This book (and others such as Bard's recent volume on morphogenesis) will help serve as a counterbalance to the prevailing direction of the field and remind us that development can be studied in more than just one way. It makes one think that perhaps what is needed is the Human Epigenome Project. We could start with simple invertebrates. . . .

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